IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Haddad, Maher M.
Avi ASHKENAZI, et al.	Group Art Unit: 1644
Application Serial No. 10/767,374	Confirmation No. 4761
Filed: January 29, 2004	Attorney's Docket No. 39780-1216 R1C1D1
For: **COMPOUNDS, COMPOSITIONS AND METHODS FOR THE TREATMENT OF DISEASES CHARACTERIZED BY A-33 RELATED ANTIGENS**	**Customer No. 35489**

DECLARATION OF MENNO VAN LOOKEREN CAMPAGNE, Ph.D. UNDER 37 C.F.R. § 1.132

I, Menno van Lookeren, Ph.D., declare and say as follows:

1. I obtained a masters degree in neuropharmacology from the State University of Utrecht, Utrecht, the Netherlands in 1987. In 1991, I was awarded a Ph.D. at the Rudolf Magnus Institute of Pharmacology, State University of Utrecht, Utrecht, the Netherlands.

2. Between 1991 and 1997 I worked as a post-doctoral fellow, first at the Netherlands Institute of Brain Research, Amsterdam, the Netherlands, and later at Hoffmann-La Roche, Department of Neuroscience, Basel, Switzerland. From 1997 to 1999 I was employed as a visiting scientist at the Department of Cardiovascular Research of Genentech, Inc., South San Francisco, CA. I am currently a scientist at the Department of Immunology of Genentech, Inc.

3. My Curriculum Vitae, including a list of my publications, is attached to and forms part of this Declaration (Exhibit A).

4. My current responsibilities include strategic leadership for initiation and advancement of discovery programs in immunology. As part of these responsibilities, I am responsible for the identification and functional characterization of novel molecules expressed on

myeloid cells and for developing therapeutic entities for the treatment of inflammatory and autoimmune diseases.

5. Experiments to test the PRO362 polypeptide in mouse models of collagen-induced arthritis (CIA) and antibody-mediated arthritis were conducted in my laboratory, either personally by me or under my supervision.

6. For use in such experiments, a murine PRO362-Ig fusion protein was generated by fusing the hinge, CH2 and CH3 domains of murine IgG1 to the extra cellular domain (aa 1-200) of murine PRO362 (muPRO362), the polypeptide sequence of which is shown in Exhibit B. murine PRO362 shows about 67% overall sequence identity to human PRO362, with about 83% identity residing in the extracellular domain. A fusion containing a double mutation preventing Fc receptor binding was used to control for Fc receptor regulation. The nucleotide sequence of the muPRO62-Fc fusion protein is shown as Exhibit C. Protein was produced in CHO cells by transient transfections of plasmid DNA. The fusion protein was purified by running the cell supernatant over a protein A column followed by ion-exchange chromatography to eliminate aggregates. Serum half life was estimated by injecting a single dose of 4 mg/kg muPRO362-Fc in a C57B6 mouse followed by obtaining serum from the mice at specified time intervals. The serum levels of muPRO362-Fc was determined by a sandwich ELISA using to anti-muPRO362 monoclonal antibodies recognizing different epitopes on the extracellular domain of muPRO362.

7. In the collagen-induced arthritis (CIA) model, 70 DBA-1J 7 to mice (7 to 8 weeks old, Jackson Laboratories) were divided into 5 treatment groups, two groups (G1 and G3) with 15 mice per group, two groups (G4 and G5) with 10 mice per group, and one group (G2) with 20 mice.

G1: MuIgG1 isotype 4 mg/kg in 100 µl saline, s.c., 3-times per week for 7 weeks (n=15).

G2 : MuPRO362-IgG1 4 mg/kg in 100 µl saline, s.c., 3-times per week for 7 weeks (n=20).

G3: MuTNFRII-IgG1 isotype 4 mg/kg in 100 µl saline, s.c., 3-times per week for 7 weeks (n=15).

G4: MuIgG1 isotype 4 mg/kg in 100 µl saline, s.c., 3-times per week for 7 weeks, anaesthesia with in vivo microCT (n=10).

G5: MuTNFRII-IgG1 1.0 mg/kg in 100 µl saline, s.c., 3-times per week for 7 weeks, anesthesia with *in vivo* microCT (n=10).

TNF is a cytokine secreted by mononuclear phagocytes, Ag-stimulated T cells, NK cells and mast cells. It is involved in normal inflammatory and immune responses. TNF-α plays an important role in the pathogenesis of rheumatoid arthritis (RA). Elevated levels of TNF were found in synovial fluid of RA patients. In this protocol, mTNFRII-Fc was used as a positive control, to block the interaction between TNF and its cell surface receptors.

All mice from G1 to G5 were immunized with 100 µg bovine collagen type II in 100 µl Complete Freund's Adjuvant (CFA) on day). The collagen type II in CFA was injected intradermally at the base of the tail on the right side. At day 21, a second immunization with 100 µg bovine collagen type II in 100 µl of incomplete Freund's Adjuvant was given intradermally at the left side of the tail.

Animals were checked daily. Mice in the G4-5 groups were anesthesized with isoflurane and in vivo microCt was performed weekly. Terminal faxitron X-Rays and microCT were taken at the end of study, ad joint lesion/erosion was evaluated.

On day 35 and at the termination of the study, mice in groups G1-5 were bled fro serum pK and anti-collagen type II antibody titer (100 µl orbital bleed). On day 70 all mice were terminally bled intracardiac under 3% isoflurane for terminal hemogram and differential leukocyte count and serum for pK (G3).

The mice were euthanized at day 70 post induction of arthritis. All four limbs were collected for radiographs, microCT and histopathology.

Figure 1 (Exhibit D) shows significant reduction in joint swelling in muPRO362-Fc treated mice.

Immunohistochemistry performed on formalin-fixed, paraffin-embedded tissue (H&E staining), obtained from muPRO362-Fc treated animals at day 70, shows inhibition of joint inflammation as a result of treatment. Figure 2 (Exhibit E) shows H&E stained sections of a meta-tarsal joint of a DBA1/J mouse 70 days after immunization with collagen type II. A. Massive inflammatory cell infiltrate is found in the areas surrounding tendon sheats and the area surrounding the joint cavity; B. Detail of A; C. Low degree of inflammatory infiltrate in the joint

of a mouse treated with muPRO362-Fc. Few inflammatory cells were found in the areas surrounding the tendon sheats and the joint cavity; D.

Figure 3 (Exhibit F) shows that cortical bone volume was preserved in joints of mice treated with muPRO362-Fc. Mice in control IgG- and muPRO362-Fc-treated groups were sacrificed 70 days after collagen injection, and joints were scanned by μCT. Bone erosion and loss of bone density in joints of mice representative of muPRO362-Fc and control IgG groups are shown in the left figure as compared to muIgG1 treated animals. Preservation of cortical bone volume was significantly greater in muPRO362-Fc treated animals. The images are a three-dimensional surface rendering created from the μCT data using Analyze image analysis software.

Figure 4 (Exhibit G) shows that muPRO362-Fc treatment does not alter the number nor the morphology of tissue resident macrophages. Livers and lungs from mice treated with either anti-gp120 IgG1 (left figures) or muPRO362-Fc (right figures) were dissected, fixed in formalin and embedded in paraffin wax. Seven micron sections were stained using an antibody to F4/80. Careful examination of the sections shows equal numbers of F4/80 positive macrophages in both treatment groups. In addition, there were no differences observed in the morphology of the macrophages

Figure 5 (Exhibit H) shows that muPRO362-Fc treatment does not affect serum anti-collagen antibody titers. Serum titers of anti collagen antibodies were determined 70 days following immunization. No differences were found in the serum titers of IgG1, IgG2a and IgM subclasses of antibodies in muPRO362-Fc treated versus anti gp120 treated animals. This means that muPO362-Fc does not affect antibody responses in mice immunized with collagen type II.

Figure 6 (Exhibit I) shows that muPRO362-Fc decreases the number of circulating inflammatory macrophages. Peripheral blood was obtained from muPRO362-Fc and anti gp-120 treated animals 70 days after immunization and analysed by flow cytometry using markers for inflammatory and non-inflammatory monocytes. MuPRO362-Fc treated animals showed a significant increase in the number of inflammatory monocytes and a decrease in the number of non-inflammatory monocytes as compared to the anti gp120 treated group.

The results of these experiments demonstrate that the muPRO362-Fc fusion protein inhibits collagen-induced arthritis. In particular, the results show that muPRO362-Fc inhibits

joint swelling, inhibits inflammation, preserves cortical joint bone volume, and decreases the number of circulating inflammatory macrophages.

7. PRO362-Fc fusion proteins were also tested in a mouse model of antibody-mediated arthritis.

Antibody-mediated arthritis can be induced by i.v. injection of a combination of four different monoclonal antibodies generated by the Arthrogen-CIA® mouse B-hybridoma cell lines (Terato et al., J. Immunol. 148:2103-8 (1992)). Three of the monoclonal antibodies recognize autoantigenic epitopes clustered within an 84 amino acid residue fragment, LyC2 (the smallest arthritogenic fragment of type II collagen) of CB11 and the fourth monoclonal antibody reacts with LyC1. All four antibodies recognize the conserved epitopes shared by various species of type II collagen and cross-react with homologous and heterologous type II collagen (Terato et al., supra; Terato et al., Autoimmunity 22:137-47 (1995)). The Arthrogen-CIA® arthritis inducing monoclonal antibody cocktail is commercially available (Chemicon International, Inc., Temecula, CA, catalog No. 90035).

In these experiments, 10 BALB-c mice(CR/Hollister) of 4-5 weeks, were divide into two groups, with 5 mice in each group.

Animals were treated daily with 100 µg muPRO362-Fc or 100 µg control-Fc (anti-gp120 IgG1), starting the day prior to the injection of the antibody cocktail (day -1), and continuing until day 14. Animals were checked at least two-times per day, and written records of observations were kept. The extent of disease was scored by visual observation.

Visual scoring system:

0 = No evidence of erythema and swelling

1 = Erythema and mild swelling confined to the mid-foot

2 = Erythema and mild swelling extending from the ankle to the mid-foot

3 = Erythema and moderate swelling extending from the ankle to the metatarsal joints

4 = Erythema and severe swelling encompass the ankle, foot and digits

Nestlets were used as an enrichment device and to provide extra padding for the animals. All animals were sacrificed on day 14, and joints were harvested for immunohistochemical staining or haematoxylin-eosin staining. Blood was sampled for hematological analysis.

Figure 7 (Exhibit J) shows macrophage infiltration in joints following antibody-induced arthritis (AIA), generated with F4/80 staining in undecalcified frozen joints. Female Balb/C mice were injected with 2 mg of anti collagen antibodies (arthrogen) i.v. followed 3 days later by injection with 25 ug LPS i.p. 14 days following antibody injection, mice were euthanized and the paws were collected, and embedded in polyvinyl alcohol. 7 μm thick sections were cut from the frozen joints and stained with antibodies to muPRO362 and to F4/80, a macrophage specific marker.

Figure 8 (Exhibit K) demonstrates that muPRO362 prevents joint swelling following antibody-induced arthritis in Balb/c mice. Arthritis was induced by the method of Terato and colleagues (Terato et al., (1992), supra; Terato et al., (1995) supra) using a mixture of 4 monoclonal antibodies recognizing a conserved epitope on callegn type II (Chemicon). Female Balb/C mice, 6 weeks old, were injected i.v. with 2 mg anti CII antibody followed 3 days later with an i.p. injection of 25 μg LPS. Animals were treated daily either with murine PRO362-Fc fusion protein or with a control-Fc fusion protein. Dosing was 4 mg/kg in 100 μl PBS subcutaneous. Treatment started the day prior to anti collagen antibody injection and continued until them ice were euthanized at day 14. Mice were observed daily post LPS injection for swelling of the hind paw as a sign of arthritis. The severety of arthritis was graded on a 1-16 scale as follows: 0 = No evidence of erythema and swelling, 1 = Erythema and mild swelling confined to the mid-foot (tarsal) or ankle, 2 = Erythema and mild swelling extending from the ankle to the mid-foot, 3 = Erythema and moderate swelling extending from the ankle to the metatarsal joints, 4 = Erythema and severe swelling encompass the ankle, foot and digits.

Therapeutic treatment was performed similar to prophylactic treatment apart from the treatment start which was at day 4 rather than day -1. muPRO362-Fc treatment reduced levels of inflammatory cytokines in paws of AIA mice. Measurement of cytokine, C3a and C5a concentration in arthritic hindpaw performed according to the method of Kagari et al, J. Immunol. 169:1459-66 (2002). In short, at the indicated time points following the induction of antibody-induced arthritis, paws were collected and frozen in liquid nitrogen. Subsequently, paws were pulverized on a liquid nitrogen-cooled metal plate and dispersed in ice-cold PBS containing 0.1% PMSF (Sigma). The samples were homogenized with a Vitatron (NL) homogenizer on ice, insoluble parts were removed by spinning at 14000 g for 10 min and

collection of supernatant. Cytokines in the supernatant were measure using cytokine ELISA's from BD Pharmingen.

muPRO362-Fc treatment inhibits deposition of complement C3 but not of IgG2a on cartilage in AIA. Female Balb/C mice were injected with 2 mg of anti collagen antibodies (arthrogen) i.v. followed 3 days later by injection with 25 ug LPS i.p. 14 days following antibody injection, mice were euthanized and the paws were collected, embedded in polyvinyl alcohol and frozen in ispenthane cooled on dry iced. 7 um thick sections were cut from the frozen joints and stained with a FITC-coupled polyclonal antibody to murine C3 (Calbiochem) and a polyclonal A594-coupled antibody to murine IgG2a (Jackson Immunoresearch). Sections were photographed in a Leitz fluorescent microcope

The results of immunohistochemistry performed with H&E staining are shown in Figure 9 (Exhibit L). Control-treated mice (muIgG1) had moderate to severe arthritis (left panel), muPRO362-treated mice has minimal to no arthritis (right panel). The results show that muPRO362 inhibits joint inflammation in antibody-induced arthritis.

In conclusion, animals treated with muPRO362-Fc had significantly reduced clinical scores as compared to animals treated with anti-gp120 IgG1. PRO362 demonstrated both prophylactic and therapeutic efficacy in this animal model. The decrease in severity of arthritis was also reflected by a decrease in inflammatory cells, especially neutrophils, in the joints. There was an increased number of neutrophils in the circulation possibly reflecting a decrease in neutrophil migration into the joint. muPRO362-Fc inhibited local IL-1β and IL-6 production in parallel with clinical manifestation of RA. muPRO362 treatment did not affect immune complex deposition, but inhibited complement C3 deposition on cartilage. The effector function was found to be independent of Fc receptor binding. huPRO362-short-Fc has also demonstrated significant prophylactic activity.

9. Both the collagen-induced arthritis (CIA) and the antibody-mediated arthritis models have been used my many laboratories to test drug candidates for the treatment of human rheumatoid arthritis. The histopathology of CIA resembles those seen in RA with synovial proliferation that progresses to pannus formation, cartilage degeneration/destruction and marginal bone erosions with subsequent joint deformities. Antibody-mediated arthritis differs from CIA in that instead of injecting the antigen (bovine collagen type II), antibodies

recognizing type II collagen are injected. In this way, adaptive B and T cell responses are circumvented to directly induce effector functions on macrophages and neutrophils through Fc receptor and complement-mediated activation. Based on the experimental findings in these two well known and recognized animal models, described in paragraphs 7 and 8 above, it is my considered scientific opinion that PRO362, a novel macrophage associated receptor with homology to A33 antigen and JAM1, is a promising drug candidate for the treatment of rheumatoid arthritis. In addition, based on these experimental data, including the demonstrated ability of PRO362 to reduce the level of inflammatory cytokines, and my knowledge of the relevant art, it is my considered scientific opinion that PRO362 has anti-inflammatory properties, which are expected to lead to the development of therapeutic approaches for the treatment other inflammatory diseases or conditions as well.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Menno van Lookeren Campagne, Ph.D.

03.14.06
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Employment and Education

1999 - present:	**Scientist**, Department of Immunology, Genentech Inc., South San Francisco, California, U.S.A.
1997 - 1999:	**Visiting Scientist**, Department of Cardiovascular Research, Genentech Inc., South San Francisco, California, U.S.A.
1994 -1997:	**Post-Doctoral Fellow**, Hoffmann-la Roche, Department of Neuroscience, Basel, Switzerland.
1991-1994:	**Post-Doctoral Fellow**, Netherlands Institute of Brain Research, Amsterdam, Netherlands.
1987-1991:	**Ph.D.** Rudolf Magnus Institute of Pharmacology, State University of Utrecht, Netherlands.
1979-1987:	**Masters** degree in neuropharmacology, State University of Utrecht, Utrecht, Netherlands.

Research Experience

1999 - present: Genentech Inc.: Provide strategic leadership for initiation and advancement of discovery programs in immunology. Responsible for the identification and functional characterization of novel molecules expressed on myeloid cells and responsible for developing therapeutic entities that target these molecules in inflammatory- and autoimmune diseases. *Reporting to Dr. Andy C. Chan and Dr. Flavius Martin.*

1997 - 1999: Genentech Inc.: Studied the molecular mechanisms of delayed neuronal death in a model of mild focal cerebral ischemia. Combined magnetic resonance imaging with novel molecular biology tools to correlate the time course of delayed neuronal death with the transcriptional activation and expression of genes involved in DNA repair and anti-oxidant activities. Supervised the development of a model of mild focal ischemia in the mouse. Knock-out and transgenic approaches have been applied to study the role of key anti-oxidant and metal-detoxifying proteins in protecting against ischemic neuronal cell death in mouse models. *Advisors: Dr. G. Roger Thomas and Dr. David G. Lowe.*

1994 -1997: Hoffmann-la Roche: Studied the role of pro-apoptotic and anti-apoptotic gene expression in delayed cell death following global and focal cerebral ischemia using biochemical, molecular biological and immunohistochemical approaches. Compared the gene expression pattern in neurons following cerebral ischemia with that in neurons undergoing apoptotic cell death during development. *Advisor: Dr. Ramanjit Gill.*

1991-1994: Netherlands Institute of Brain Research: Performed studies on the physiology and pathophysiology of excitatory amino acids and their antagonists in the developing rat brain. These studies were performed to obtain a better understanding of the mechanisms and possible treatment strategies of neonatal asphyxia/ischemia. Magnetic resonance imaging and electrophysiology were

applied to detect early cellular pathology following injection of excitatory amino acids. Receptor-ligand binding essays were performed to study conformational changes in excitatory amino acid receptors during normal development and following blockade of the receptors using specific antagonists. In addition, the induction of apoptosis by excitatory amino acids in the developing rat brain was directly addressed using several techniques including *in situ* end-labeling, DNA extraction and electron microscopy. *Advisors: Dr. Robert Balázs and Dr. Klaas Nicolay.*

1987-1991: University of Utrecht: Developed several new methods to localize neuronal proteins at the electron microscopic level. Used this approach to study the role of the growth-associated protein B-50/GAP-43 in neuronal survival, plasticity and differentiation in cell cultures and in the developing rat brain. *Thesis advisors: Prof. Dr. Willem-Hendrik Gispen and Prof. Dr. Arie J. Verkley.*

Research Interest

Identifying novel therapeutic targets for development of drugs to treat autoimmune and anti-inflammatory diseases.

Ad Hoc Reviewer and Referee

Journal of Neurocytology (1992)
Neuroreport (1993)
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Van Bruggen, N. and **Van Lookeren Campagne, M.**, *Dynamics of cerebral tissue injury and perfusion after temporary hypoxia-ischemia in the rat.* Stroke 29 (1998) 704 (editorial comment)

Van Lookeren Campagne, M. and Gill, R. *DNA fragmentation associated with chromatin condensation is a late consequence of ischaemic cell death rather than a hallmark of apoptosis.* In: Pharmacology of Cerebral Ischemia, J. Kriegelstein (Ed.) Raven Press, New York (1996) pp. 77-83.

Van Lookeren Campagne, M., Oestreicher, A.B., De Graan , P.N.E. and Gispen, W.H. *Role of B-50/GAP43 in nerve growth cone function.* In: The Nerve Growth Cone, P.C. Letourneau, S.B. Kater and E.R. Macagno (Eds.), Raven Press, New York, pp. 97-109 (1992).

Awards

1993	Dutch Academy of Science Student Exchange Fellowship
1994	European Community Short Term Fellowship
1996	Aaron Diamond Post-doctoral Fellowship (declined)

Committee Member

University of Oslo, Insititute of Anatomy. Committee member and second opponent in examination for the degree of Doctor of Medicin, Erlend Nagelhus. Thesis: *Water and Volume Homeostasis in the Central Nervous System: Role of Glial Cells*, December 1998.

University of Kuopio, Finland. Committee member and first opponent in examination fro the degree of Doctor of Philosophy, Kaisa Kurkinen. Thesis: The role of PKCdelta in cerebral ischemia, December 2002.

Sequence 8

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Sequence 17

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Sequence 17

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